

# SUSCEPTIBILITY OF STRICT AND FACULTATIVE ANAEROBES ISOLATED FROM ENDODONTIC INFECTIONS TO METRONIDAZOLE AND $\beta$ -LACTAMS

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**Received:** December 13, 2006 - **Modification:** April 17, 2007 - **Accepted:** August 27, 2007

## ABSTRACT

**E**ndodontic infections are mixed aerobic-anaerobic infections and several microbial groups associated to these pathologies are also involved in orofacial infections. The goal of this study was to evaluate the susceptibility of microorganisms isolated from endodontic infections to  $\beta$ -lactams and metronidazole and verify the production of  $\beta$ -lactamases. Clinical specimens were collected from 58 endodontic infections of 52 patients. The microorganisms were isolated in selective and non-selective culture media, under anaerobiosis and aerobiosis, and identified using biochemical methods. In the susceptibility tests, it was used an agar dilution method, and Wilkins-Chalgren agar enriched with blood, hemin and menadione for the anaerobes, while Mueller-Hinton agar was employed for the facultative anaerobes. The production of  $\beta$ -lactamases was evaluated through the biological and chromogenic cephalosporin methods. All tested isolates were sensitive to imipenem and 99.3% to amoxicillin/clavulanate association, while 16.1% showed resistance to amoxicillin and penicillin G, and 4.89% to cefoxitin. Resistance to metronidazole was just found in facultative anaerobes. Production of  $\beta$ -lactamases was detected in 18.2% of the isolates and presented a correlation with resistance to  $\beta$ -lactams.

**Uniterms:** Infection; Antibacterial agents;  $\beta$ -lactams.

## INTRODUCTION

Endodontic infections are mixed aerobic-anaerobic infections characterized by a correlation between the virulence of specific microorganisms and the host's immune system<sup>23</sup>. In the pathogenesis of these infections, specific microbial groups play a relevant role, particularly strict anaerobes, which are also associated to their clinical symptomatology<sup>8,10</sup>. Such mixed infections may afford an optimum situation for the exchange of genetic elements between bacterial species.

The clinical outcome of endodontic treatment depends on controlling the microbial biofilm that is established inside the root canal system by means of instrumentation and antibacterial irrigation. However, residual microorganisms in the root canals are frequently involved in orofacial infections<sup>25</sup>. In the treatment of these infections, systemic antibiotics can be prescribed to prevent the spread of infection and onset of serious complications<sup>3</sup>. However, empirical prescription of antimicrobials as part of endodontic management is problematic,

given the diversity of potential pathogens and their different sensitivity drugs.

The determination of *in vitro* antimicrobial susceptibility may be important in certain situations, for example, to monitor patterns of susceptibility and resistance in the population and to aid in the selection of an appropriate antibiotic when indicated in the endodontic treatment<sup>17</sup>. Moreover susceptibility tests for anaerobes are not routine in Brazil. Therefore, the objective of this study was to evaluate the susceptibility of bacteria isolated from endodontic infections to  $\beta$ -lactams and metronidazole and evaluate the production of  $\beta$ -lactamases.

## MATERIAL AND METHODS

This study was approved by the Ethics in Human Research Committee of the Dental School of Araçatuba (FOA 01638).

## Patients

The population of this study consisted of 33 patients, presenting at the Dental School of Araçatuba of São Paulo State University (UNESP), and 19 patients attending private clinics for endodontic treatment. Medical histories revealed that all patients were in good general health. Patient ages ranged from 19 to 61 years (mean age = 31.5). At the first visit, written informed consent was obtained from each patient before enrolment in the study. Patients who had received antibiotics 3 months before the root canal therapy and teeth with a periodontal pocket probing depth greater than 4 mm were excluded.

## Clinical Signs and Symptoms

It was recorded the presence of carious tissue, pulp status, presence and clinical conditions of restorations, pain on occlusion, tenderness to percussion or palpation, swelling and presence of periapical radiolucency, mobility, periodontal pocket probing depth, presence of a sinus of endodontic origin, intracanal condition, such as a dry canal or the presence of exudates<sup>10</sup>. The diagnosis of endodontic pathology was performed as described by Sigurdsson<sup>26</sup>.

## Clinical Samples

The specimens were taken from 58 endodontic infections of 52 patients. Clinical samples were collected from 32 teeth with chronic endodontic infection, 10 teeth presenting acute endodontic infection with acute periapical abscess and from 16 teeth presenting exacerbated periapical lesions and pulpal necrosis. After anesthesia, a rubber dam was placed. The operative field was disinfected and antisepsis of tooth enamel was performed<sup>10</sup>. Access to the root canal system was gained with sterile burs. In addition, sterile saline delivered through a manual irrigation to avoid contamination from dental unit waterlines. After removal of carious dentin, canal patency was established with minimal instrumentation and without use of any chemically active irrigant. Thereafter, the canal was soaked with VMGAIII transport solution and clinical specimens were collected by using sterile paper points, which were introduced into the full canal length, as previously determined with a preoperative radiograph. The paper points were kept in place for 30-60 seconds and were transferred to VMGA III.

In the laboratory, aliquots of VMGA III were submitted to serial 10 fold dilutions and were plated on Fastidious Anaerobic Agar (FAA) supplemented with yeast extract (0.5%), hemin (5  $\mu$ g/mL), menadione (1  $\mu$ g/mL) and 5% horse blood, crystal violet erythromycin agar (CVEA), FAA + nalixidic acid (0.001% w/v) + vancomycin (0.5mg/L) and 5% horse blood, FAA + vancomycin (0.5 mg/L) and kanamycin (0.016 mg/L), and incubated under anaerobiosis (90% N<sub>2</sub> + 10% CO<sub>2</sub>), at 37°C, for 14, 04, 04 and 14 days, respectively<sup>11</sup>. The same dilutions were plated on horse blood Brain Heart Infusion Agar and on MacConkey agar, incubated aerobically, at 37°C for 2 days.

Pure cultures were obtained and the isolates were subjected to morphocellular and morphocolonial analyses, respiratory test and catalase assay. This was followed by identification of the isolates at the genus level and, whenever possible, at the species level, using commercial kits<sup>10</sup> (BioMérieux SA, Marcy-

l'Etoile, France) and carbohydrate fermentation.

## Microorganisms

A total of 119 isolates of strict and facultative anaerobic bacteria were subjected to the susceptibility tests. The bacterial inocula were standardized in 10<sup>5</sup> cells and transferred to the Petri plates containing the antimicrobials and the control plates (without drugs), using a Steer's replicator (Cefar Ltda, São Paulo, SP, Brazil). The minimum inhibitory concentration (MIC) was defined as the smallest concentration of the drug able to completely inhibit the bacterial growth.

*F. nucleatum* ATCC 10953 and ATCC 25586, *E. lentum* ATCC 43055, and *B. fragilis* ATCC 23745 were used as reference strains for quality control in the assays involving strict anaerobes, while *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212 were used in the assays involving facultative anaerobes. The test and control dishes were incubated anaerobically (90% N<sub>2</sub> + 10% CO<sub>2</sub>) or aerobically at 37°C, for 48 hours.

## Antimicrobial susceptibility tests

The following antimicrobial drugs were tested: amoxicillin (Bayer S.A., São Paulo, SP, Brazil), amoxicillin/clavulanic acid (Smithkline Beecham Brasil Ltda, São Paulo, SP, Brazil), cefoxitin (Merck Sharp & Dohme, São Paulo, SP, Brazil), imipenem (Merck Sharp & Dohme, São Paulo, SP, Brazil), benzylpenicillin (Fontoura-Wyeth S.A., São Paulo, Brazil) and metronidazole (Laboratórios Pfizer Ltda, Guarulhos, SP, Brazil). The breakpoints used for interpretation were generally those recommended by the NCCLS<sup>20</sup>. Antimicrobials were tested in concentrations that ranged from 0.06  $\mu$ g/mL to 512  $\mu$ g/mL. The tests were carried out by agar dilution method. Wilkins-Chalgren agar supplemented with horse blood, hemin (5  $\mu$ g/mL) and menadione (1  $\mu$ g/mL) was used for strict anaerobes and Mueller-Hinton agar was used for facultative anaerobes.

## Detection of $\beta$ -lactamases

All microorganisms able to resist at least 0.5  $\mu$ g/mL of any of the tested  $\beta$ -lactams were submitted to tests to evaluate the production of  $\beta$ -lactamases, using biological<sup>24</sup> and chromogenic cephalosporin methods<sup>6</sup>. These two methods were performed because nitrocefin-based  $\beta$ -lactamase assays have not proven useful in detecting  $\beta$ -lactamase production by some anaerobes<sup>6</sup>.

A chromogenic cephalosporin  $\beta$ -lactamase assay using Cefinase disks was performed according to the manufacturer's instructions. Briefly, 6-mm-diameter filter paper disks impregnated with nitrocefin were moistened with 0.85% NaCl, and several isolated colonies of test organisms were transferred to the disk. Disks were examined for the appearance of a pink-red coloration for up to 10-60 min<sup>6</sup>.

In the biological method<sup>24</sup>, 20  $\mu$ L of cultures of the resistant isolate were plated on the surface of Wilkins-Chalgren agar, containing 0.5  $\mu$ g/mL of the tested  $\beta$ -lactam. These plates were then incubated anaerobically or aerobically at 37°C for 48 h. After this incubation period, the cultures were exposed to chloroform fumes for 20 min. and then covered with 3 mL of semi-solid brain heart infusion (BHI) agar (0.7% agar) previously inoculated with 10<sup>6</sup> cells of *Streptococcus pyogenes* FOA-94F14

sensitive to all tested  $\beta$ -lactams in a concentration of  $\leq 0.06 \mu\text{g/mL}$ . The Petri dishes were then incubated under aerobiosis for 24 h at  $37^\circ\text{C}$ . After incubation, presence or absence of growth of the streptococcal strain was checked. The presence of this halo of growth was indicative of the degradation of the  $\beta$ -lactam. *Bacteroides fragilis* ATCC 43858 was used as the positive control of  $\beta$ -lactamase production.

## RESULTS

Susceptibility to antimicrobial agents was evaluated on two isolates of *Actinomyces naeslundii*, four isolates of *A. israelii*, one isolate of *A. odontolyticus*, two isolates of *Actinomyces* sp., four *Bacteroides* sp., three isolates of *Eubacterium* sp., one isolate of *E. lentum*, five *Enterococcus faecalis* and *Fusobacterium* sp., twelve *F. nucleatum* and *Peptostreptococcus* sp., three *P. anaerobius*, two *P. asaccharolyticus*, four isolates of *P. micros*, three *Porphyromonas gingivalis*, five *Prevotella* sp., ten isolates *P. intermedia-nigrescens*, seven *Propionibacterium* sp., three *Proteus* sp. and *Selenomonas* sp., nineteen isolates of *Streptococcus* spp., four *Staphylococcus epidermidis*, and five isolates of *Veillonella* sp.

The data presented in Table 1 show that imipenem was the most effective  $\beta$ -lactam, while variable levels of microbial resistance were observed for all other drugs. The association amoxicillin/clavulanic acid was effective against all isolates but one strain of *Proteus* sp., which produced  $\beta$ -lactamases active on amoxicillin, penicillin G, and cefoxitin and was not inhibited by clavulanic acid. Resistance to  $\beta$ -lactams was high among the Gram-negative anaerobes, such as *Prevotella intermedia-nigrescens* group and *Veillonella* sp. Resistance to metronidazole was confined to facultative anaerobes, although some Gram-positive strict anaerobes of genera

*Peptostreptococcus* and *Bacteroides* presented an intermediate susceptibility.

Resistance to benzylpenicillin and amoxicillin varied among the clinical strains, but most values of minimal inhibitory concentrations were closely related and showed the same pattern of  $\beta$ -lactamase production. Strains able to hydrolyze penicillin G were able to perform amoxicillin degradation. Ten (62.5%) out of sixteen isolates resistant to benzylpenicillin and amoxicillin were  $\beta$ -lactamase producers, while all strains resistant to cefoxitin were able to produce these hydrolytic enzymes.

$\beta$ -lactamase detection using biological and nitrocefin methods provided the same results, which are presented in Table 2. There was a correlation between resistance to  $\beta$ -lactams and production of  $\beta$ -lactamases, which were active on penicillins and cephalosporins. Figure 1 presents the data referring to microbial resistance to  $\beta$ -lactams and  $\beta$ -lactamase production. There was a close correlation between resistance to  $\beta$ -lactams and production of  $\beta$ -lactamases. In strict anaerobes, 70% of all isolates resistant to  $\beta$ -lactams were  $\beta$ -lactamase producers, while 80% of all facultative isolates resistant to these drugs also produced such enzymes.

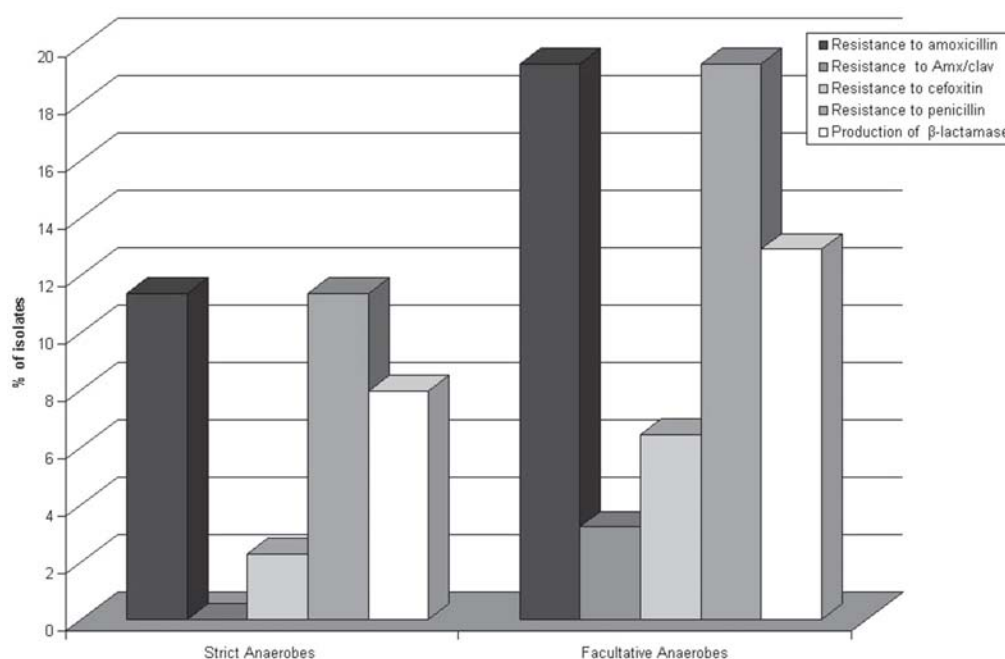


FIGURE 1- Resistance of strict and facultative anaerobes to  $\beta$ -lactams and production of  $\beta$ -lactamases

**TABLE 1-** Resistance of 119 clinical isolates isolated from endodontic infections to metronidazole and  $\beta$ -lactams

Isolate (tested number) and antimicrobials	<sup>1</sup> S	% <sup>2</sup> I	<sup>3</sup> R	Range	MIC( $\mu$ g/mL) <sup>4</sup> MIC <sub>50</sub>	<sup>5</sup> MIC <sub>90</sub>
<i>Actinomyces</i> spp. (9)						
benzylpenicillin	66.67	22.22	11.11	$\leq 0.006$ -32	0.125	32
amoxicillin	66.67	22.22	11.11	$\leq 0.006$ -32	0.125	32
amoxicillin/clavulanic acid	100	0.0	0.0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
cefoxitin	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
imipenem	100	0.0	0.0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
metronidazole	66.67	33.33	0.0	$\leq 0.006$ -16	0.5	16
<i>Bacteroides</i> sp. (4)						
benzylpenicillin	75	0.0	25	$\leq 0.006$ -256	0.25	256
amoxicillin	75	0.0	25	$\leq 0.006$ -128	0.25	128
amoxicillin/clavulanic acid	100	0.0	0.0	$\leq 0.006$ -1	0.25	1.0
cefoxitin	75	0.0	25	$\leq 0.006$ -32	0.125	32
imipenem	100	0.0	0.0	$\leq 0.006$ -8	0.125	8
metronidazole	100	0.0	0.0	$\leq 0.006$ -16	0.125	16
<i>Eubacterium</i> spp. (4)						
benzylpenicillin	100	0	0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
amoxicillin	100	0	0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
amoxicillin/clavulanic acid	100	0	0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
cefoxitin	100	0	0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
imipenem	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.125
metronidazole	100	0.0	0.0	$\leq 0.006$ -1	$\leq 0.006$	1
<i>Enterococcus faecalis</i> (5)						
benzylpenicillin	80	0.0	20	$\leq 0.006$ -128	$\leq 0.006$	128
amoxicillin	80	0.0	20	$\leq 0.006$ -128	0.125	128
amoxicillin/clavulanic acid	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
cefoxitin	80	0.0	20	$\leq 0.006$ -128	$\leq 0.006$	128
imipenem	100	0.0	0.0	$\leq 0.006$ -2	0.125	2
metronidazole	0.0	0.0	100	32->512	128	>512
<i>Fusobacterium</i> spp. (17)						
benzylpenicillin	70.59	11.76	17.65	$\leq 0.006$ -256	0.125	8
amoxicillin	70.59	11.76	17.65	$\leq 0.006$ -256	0.125	8
amoxicillin/clavulanic acid	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
cefoxitin	100	0.0	0.0	$\leq 0.006$ -8	$\leq 0.006$	4
imipenem	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
metronidazole	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
<i>Peptostreptococcus</i> spp. (21)						
benzylpenicillin	85.72	9.52	4.76	$\leq 0.006$ -16	$\leq 0.006$	0.5
amoxicillin	90.48	4.76	4.76	$\leq 0.006$ -16	$\leq 0.006$	0.5
amoxicillin/clavulanic acid	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
cefoxitin	100	0.0	0.0	$\leq 0.006$ -2	$\leq 0.006$	1
imipenem	100	0.0	0.0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
metronidazole	90.48	9.52	0.0	$\leq 0.006$ -16	0.125	2.0
<i>Porphyromonas gingivalis</i> (3)						
benzylpenicillin	100	0	0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
amoxicillin	100	0	0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
amoxicillin/clavulanic acid	100	0	0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
cefoxitin	100	0	0	$\leq 0.006$ -0.5	$\leq 0.006$	0.5
imipenem	100	0	0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25
metronidazole	100	0	0	$\leq 0.006$ -0.25	$\leq 0.006$	0.25

Cont. Table 1

<i>Prevotella</i> spp. (15)						
benzylpenicillin	60	20	20	≤0.006-512	0.25	128
amoxicillin	66.67	13.33	20	≤0.006-256	0.25	128
amoxicillin/clavulanic acid	93.33	6.67	0.0	≤0.006-2	0.125	2
cefoxitin	80	13.34	6.67	≤0.006-128	0.5	32
imipenem	100	0	0	≤0.006-1	≤0.006	1
metronidazole	100	0	0	≤0.006-0.5	≤0.006	0.5
<i>Propionibacterium</i> sp. (7)						
benzylpenicillin	85.71	14.28	0.0	≤0.006-16	≤0.006	1
amoxicillin	85.71	14.28	0.0	≤0.006-16	0.125	1
amoxicillin/clavulanic acid	100	0.0	0.0	≤0.006-0.5	≤0.006	0.5
cefoxitin	100	0.0	0.0	≤0.006-2	≤0.006	1
imipenem	100	0.0	0.0	≤0.006-0.5	≤0.006	0.5
metronidazole	57.14	48.86	0.0	≤0.006-16	2	16
<i>Proteus</i> sp. (3)						
benzylpenicillin	0.0	33.33	66.67	1-256	64	256
amoxicillin	0.0	33.33	66.67	1-256	64	256
amoxicillin/clavulanic acid	0.0	66.67	33.33	4-32	4	32
cefoxitin	0.0	33.33	66.67	32-256	128	256
imipenem	66.67	33.33	0.0	2-8	2	8
metronidazole	0.0	0.0	100	>512	>512	>512
<i>Selenomonas</i> sp. (3)						
benzylpenicillin	100	0	0	≤0.006-0.5	≤0.006	0.5
amoxicillin	100	0	0	≤0.006-0.25	≤0.006	0.25
amoxicillin/clavulanic acid	100	0	0	≤0.006-0.25	≤0.006	0.25
cefoxitin	100	0	0	≤0.006-0.5	≤0.006	0.5
imipenem	100	0	0	≤0.006-0.25	≤0.006	0.25
metronidazole	100	0	0	≤0.006-0.25	≤0.006	0.25
<i>Streptococcus</i> spp. (19)						
benzylpenicillin	84.21	10.52	5.26	≤0.006-16	≤0.006	1
amoxicillin	89.48	5.26	5.26	≤0.006-16	≤0.006	1
amoxicillin/clavulanic acid	100	5.26	0	≤0.006-0.25	≤0.006	0.25
cefoxitin	89.47	10.52	0	≤0.006-32	0.25	32
imipenem	100	0	0	≤0.006-0.25	≤0.006	0.25
metronidazole	21.06	10.52	68.42	1->512	≤0.006	128
<i>Staphylococcus epidermidis</i> (4)						
benzylpenicillin	0,0	50	50	1->512	1	>512
amoxicillin	0,0	50	50	1->512	1	>512
amoxicillin/clavulanic acid	50	50	0,0	0.5-2	0.5	2
cefoxitin		75	25	2-128	2	128
imipenem	75	25	0,0	0.5-2	0.5	2
metronidazole	0,0	0,0	100	256->512	>512	>512
<i>Veillonella</i> sp. (5)						
benzylpenicillin	60	20	20	≤0.006-16	0.25	1
amoxicillin	60	20	20	≤0.006-16	0.25	1
amoxicillin/clavulanic acid	100	0	0	≤0.006-0.25	≤0.006	0.25
cefoxitin	100	0	0	≤0.006-0.5	≤0.006	0.5
imipenem	100	0	0	≤0.006-0.25	≤0.006	0.25
metronidazole	100	0	0	≤0.006-0.25	≤0.006	0.25

<sup>1</sup>Susceptible<sup>2</sup>Intermediate<sup>3</sup>Resistant<sup>4</sup>Minimal Inhibitory Concentration for 50% of the isolates<sup>5</sup>Minimal Inhibitory Concentration for 90% of the isolates



**TABLE 2-** Production of  $\beta$ -lactamases by resistant microorganisms isolated from endodontic infections

Microorganisms	Resistant isolates/ resistant isolates producers of $\beta$ -lactamases		
	Amoxicillin	Cefoxitin	Penicillin
<i>Actinomyces</i> spp.	1/0	0/0	1/0
<i>Bacteroides</i> sp.	1/1	1/1	1/1
<i>E. faecalis</i>	1/1	1/1	1/1
<i>Fusobacterium</i> spp.	3/2	0/0	3/2
<i>Peptostreptococcus</i> spp.	1/0	0/0	1/0
<i>Prevotella</i> spp.	3/3	1/1	3/3
<i>Proteus</i> sp.	2/1	1/1	2/1
<i>Streptococcus</i> spp.	1/1	0/0	1/1
<i>S. epidermidis</i>	2/1	0,0	2/1
<i>Veillonella</i> sp.	1/0	0/0	1/0
Total	16/10	4/4	16/10

## DISCUSSION

Most studies that deal with the emergence of antimicrobial resistance of oral bacteria have been done in the western world and little information is available from developing countries<sup>1</sup>, which have socioeconomic and behavioral factors that promote bacterial resistance<sup>22</sup>, such as self-prescription and misuse. The resistance of strict and facultative anaerobic microorganisms has expanded considerably<sup>2,27</sup>, which has rendered traditional prescription habits critical in the absence of laboratorial exams. This fact is further aggravated when one considers the relevance of the phenomenon of self-medication in Brazil, which limits the efficacy of treatment and can make prescribing a difficult task, as it modifies the local patterns of susceptibility to drugs.

The use of antimicrobial drugs may constitute an important adjuvant in the treatment of endodontic and periapical infections, particularly in acute cases<sup>16</sup>, but it sometimes comes up against the problem of selection of microorganisms resistant to the most frequently used drugs. Moreover, this resistance is usually manifested by the production of enzymes that are able to degrade these drugs<sup>19</sup>, and anaerobic genera, such as *Fusobacterium* and *Prevotella*, are generally the main producers of these enzymes<sup>14,15,21</sup>, but they are highly sensitive to metronidazole<sup>1</sup>. The results listed in Table 1 are in accordance with these concepts, as resistance to most  $\beta$ -lactams seems to be frequently found in some genera, such as *Prevotella* and *Fusobacterium* and facultative rods and cocci.

The resistance to amoxicillin and penicillin G was significantly higher than previously reported in the literature concerning anaerobes from oral samples retrieved in developed countries<sup>7,18,27</sup>, but the data were in accordance with data from developing countries<sup>1,3</sup>. Regarding the most frequently isolated facultative bacteria, genus *Streptococcus*, the resistance to  $\beta$ -lactams are slightly more elevated than those previously reported<sup>4,5</sup>. Thus, the bacteria that accounted for the significantly increased proportion of resistance to amoxicillin and penicillin G in clinical samples were Gram-negative rods that inhabit periodontal pockets and gingival crevice<sup>7,9</sup>.

As the combination of amoxicillin/clavulanic acid was effective on almost all the isolates resistant to the other  $\beta$ -lactams (except for one isolate of *Proteus* sp.), as previously

observed<sup>2,11,13,19</sup>, it could be suggested that the mechanism of resistance to these drugs occurred due to the production of inactivating enzymes, although the action of some  $\beta$ -lactamases is not inhibited by addition of clavulanic acid<sup>13,18</sup>. In anaerobic Gram-negative bacteria, these  $\beta$ -lactamases are active on penicillins and cephalosporins<sup>27</sup>, as in accordance with the data presented in Table 2. On the other hand, the efficiency of imipenem on these bacteria highlights the need to leave the carbapenems for exclusive use in serious infections to avoid the dissemination of resistance.

The data shown in Table 1 substantiated the idea that clavulanic acid, a  $\beta$ -lactam devoid of antimicrobial activity but able to inhibit the action of an extensive group of enzymes that inactivate these drugs, and its associations with other  $\beta$ -lactams, may constitute an important option for prescription, particularly when the patient has a previous history of use of drugs of this group in the 2-3 preceding months.

In anaerobic Gram-negative bacteria, these  $\beta$ -lactamases are active on penicillins and cephalosporins<sup>27</sup>, as in accordance with the data presented in Table 2. Moreover, some isolates of genus *Bacteroides* and facultative anaerobes were resistant to amoxicillin, penicillin G and cefoxitin, the use of cephalosporins in substitution of penicillins in the treatment of serious mixed infections is compromised, especially in patients with history of previous use of penicillins. On the other hand, the efficiency of imipenem on these bacteria highlights the need to leave the carbapenems for use exclusively in serious infections to avoid the dissemination of resistance.

Some isolates that are resistant to  $\beta$ -lactams were proved not to be producers of  $\beta$ -lactamase by the methodology employed in this study (Table 2). This suggests that these bacteria are producers of non-exportable  $\beta$ -lactamases, as previously reported in Gram-negative bacteria<sup>12</sup>, or the existence of other mechanism of microbial resistance, such as alteration of structure of penicillin-binding proteins reducing the affinity to the  $\beta$ -lactams, or the method's sensitivity did not allow for the detection of these enzymes.

## CONCLUSIONS

The results of the present study indicate that, among the tested microorganisms, resistance to  $\beta$ -lactams was not disseminated and was more frequently found in some Gram-negative anaerobic rods, enteric microorganisms and facultative cocci. The resistance to these drugs was closely related to the production of  $\beta$ -lactamases. Resistance to metronidazole was observed only in facultative anaerobes.

## ACKNOWLEDGEMENTS

This study was partially supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo; Grants 98/6555-2 and 03/12763-7).

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